NATIONAL
42-383
Made in U. S. A.

SV Transformed cells for deter. mRNA annealing E fragments

1. SV-B3T3 Cl 6A
2. SV-B3T3 Cl 5A
3. UV-15 Cl 5
4. T2
5. SV 3T3 9/8a
6. SV 3T3 Cl 6

Antibody ag 1) Infected BSC-1
2) SV-B-3T3

Idea: To identify SV40 proteins in productively infected & transformed cells, ppt. the ^{35}S labeled proteins in these cells w antisera made ag. inf. BSC-1 (early w ara C & late) & SV-Ball 3T3.
Each serum is to be absorbed w uninf. or untr. cell extract. Imm. precipitate is washed & taken up w SDS-ME-EDTA & electrophoresed.
Control = uninf or untr. cell extr.
ppted w same antiserum.

Cell extracts for immunization:

1. Normal BSC-1
2. Inf. BSC-1 — ara C = early pr.
3. Inf. BSC-1 — 48 hrs = early + late
4. Normal Ball/3T3
5. SV transformed Ball/3T3 — T2

Note: If all genes are expressed in #3 this serum may be okay for testing all other cell extracts!

C-190

Liver RNA polymerase

SUBJECT

SHEET NO.

BY

DATE

1 gm
3 ml
6 ml
9 ml
3
12 ml

25
6
150

30 g. of liver from
washed & minced in sol'n A at 0°
+ 60 ml sol'n A - homogenized in P-E c Teflon pestle.
10-15 strokes at ~ 2000 rev/min.
Filtered through cheesecloth.
Volume made to 150 ml c sol'n A & layered
onto sol'n B - 25 ml onto 5 ml B in SW 25 rotor.
Cent at 22,000 rpm for 1 hr x 4. Pour off supe & wipe tube.
Suspend pellets in total of 10 ml of E & sonicate
for 6 - 15 sec. periods. Cent at 80,000 g for 45'.

C-190 9/16/69 Cellulose phosphate

SUBJECT

SHEET NO.

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Whatman # 2111 P11

Medium fibrous powder

Nominal total capacity 7.4 m Eq/g

Washed ē 0.5 N NaOH water, HCl water

Equil. ē 0.05 M Tris HCl pH 7.8

C-190

9/15/69 Liver RNA polymerase

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A. 0.25 M sucrose, 0.05 M Tris Cl 7.5, 0.025 M KCl, 0.005 M $MgCl_2$ (TKM), 0.0015 M $CaCl_2$

B. 2.3 M sucrose in TKM + Ca^{++}

C. 2.2 M sucrose, 0.001 M $MgCl_2$

D. 0.32 M sucrose 0.001 M $MgCl_2$, 0.02 M Tris 7.5

E. 0.05 M Tris Cl 7.8, 30% glycerol, 0.005 M ME

Stocks:

1 M Tris 7.5 - 500 ml

1 M Tris 7.8 - 500 ml

1 M $MgCl_2$ - 100 ml

1 M KCl - 1000 ml

1M = 34.2%

230

2.5 25

342

2.3

1026

684

78 6.68/10

15 1.7 34/200

342

2.2

684

684

752

151.7

C-190 10/28/69

SUBJECT

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3T3
SV 3T3 } fr K K Takemoto

1 small flask SV 3T3
1 large " 3T3

Med. changed 10/27 MEM ē 15% FBS
Next day trypsinized ē 0.5% tryp in PBS — 30'
Centif + wash ē 1 ml med.
Suspend SV 3T3 in 4 ml
3T3 in 8 ml
take 0.8 of each + 4 ml med to each of 2 flasks
Remainder + 0.6 + 1.6 50% glycerol, keep
+ put at 4° overnight (10/28-29)

BBL, Cat. #40602, Anti-Rabbit Globulin, Fluorescein labeled, Lot #9061907, has been tested in an indirect staining system employing Salmonella "O" Group D antiserum prepared in rabbits and a Salmonella typhi antigen. Satisfactory results were demonstrated at a conjugate dilution of 1:40.

These results were obtained employing a Zeiss Standard RA binocular Fluorescent Microscope, equipped with a BG-12 exciter filter and a Zeiss 50 eyepiece barrier filter. An HBO-200 mercury burner in a Zeiss housing served as the light source.

It is suggested that each laboratory determine the optimal staining titer under its own standard operating procedures.

BBL, Division of BioQuest
Cockeysville, Maryland

C-190 12/11/69 SV40 DNA f. SV inf CV1

SUBJECT

SHEET NO.
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9 – 100mm dishes f. Wed – confluent CV1
Trypsinize + suspend cells in
200 ml MEM ± 10% FBS
(Cell count = 64 per 8 small squares
= 80/cu mm or 1.6×10^7 total or 8×10^5/10 ml)
Dispense 10 ml into dishes
15 ml into 1 flask
(Avg pl ∴ had $\frac{1.6 \times 10^7}{9} \approx 2 \times 10^6$/plate)

12/12 Cells nearly confluent

12/13 Wash cells once ± PBS
(5×10^5 pfu/ml SPV)

3×10^6 (10 45 AM) I – Infect ± SV40 0.15 ml of – 16 plates
3×10^7 moi = 30
1.5×10^8 Un – Add 0.15 ml medium – 2 plates

1.5×10^8 12 45 after 1½ hrs at 37° add 10 ml regular MEM ± 10% FBS
to group I-A – 10 plates
To I-B+C add MEM ± 10% dial. FBS – 10 plates
To Un add 10 ml " " " "

12/14 Change all media except I-A
10 45 AM → Add C-thymidine to I-B + Un-B 1:5 .15 ml
24 hrs 100 μc/ml 50 μc/.205 ml (3 μc)
Add H-thymidine to I-C + Un-C .05/ml
1 mC/ml 5 mC/.0675 ml NET027X CH3 Lab (50 μc)

I-A inj – no radioactivity
I-B inj + ^{14}C-TdR
I-C inj + ^{3}H-TdR
Un-B uninj + ^{14}C-TdR
Un-C uninj + ^{3}H-TdR

12 hrs. Remove med. + wash cells × 2 c 5 ml
Tris-saline (per L 0.1 g $MgCl_2 \cdot 6H_2O$; 0.1 g $CaCl_2$;
8 g NaCl; 0.38 g KCl; 0.1 g Na_2HPO_4;
3 g Tris – adj to pH 7.4)

To each dish add 1 ml 0.6% SDS – 0.001 M EDTA
pH 7.5. after 10-20' at rm temp
scrape lysate c rubber policeman + pour
into plastic cent. tube – 8 mm diam.
Add 5 M NaCl to ⇒ 1 M + slowly
invert 10 ×. store at 4° for ≥ 8 hrs.

Cent. at 12,500 rpm (~17,000 g) × 30' in cold.
Super removed c pasteur pip to glass tubes. Keep pellets in refrig.
Count 50 l of each super except A – filter paper c
cold thymidine + Y-RNA carrier. Cold TCA wash.
Store super in refrig overnight.

C-190 12/17 SV40 DNA con'd. SHEET NO.

SUBJECT BY

DATE

Layers extr. 2x c phenol sat c 1M Tris HCl pH 8.0
then 1x c chloroform - isoamyl alc (24:1).
(Sample C lost in centrifuge).

Layers dial ag 1X SSC c .001 M EDTA .01 M Tris 7.4
Overnight in cold.
.010 ml B taken for cts.

CsCl run

	Tube 1	2	3
	A	A	B
Wt soln	2.7584 g	2.9967	3.1928 g
Eth Br	0.3 g 200 s/ml	0.3	0.3
CsCl	2.84 g	3.11 g	3.30 g
CsCl soln	0.75 ml	0.46 ml	0.21 ml
48.58% w/w			

Dens. 1.572 by weight
Adjusted by adding .02 ml water each tube
Spin in SW 50.1 at 45000 rpm x 48 hrs. at 34°F Temp. setting

From Table

ref index	1.3860	=	1.5522 g/ml	=	48.00% w/w
	1.3891	=	1.5874 g/ml	=	50.00 "
by interpol	1.3869		1.565 ?	=	48.58 "

1.572
1.566
.006

7.80 →
.0038
.027

1.3869

			% by wt.
1.3860 –	743.7 g/L	1.5522	48.00
1.3891 –	792.3	1.5874	50.00
0031		.0352	2.00
			0.58
			100.00

1.3869

48.58 g
51.42

Water

2.89
.234
3.124

3.11
.234
3.344

.01
3.30
.234
3.534

1.572

1.5660 (1.566)
1.5522
.0138

Trial – ~~13.2358~~ 3.5

.75

4.85
.234
5.084

Beaker 27.0402

.75 48 1.17
5.95
7.12

(.3) # 1 0.7422 2 0.7421 3 0.7350
+ ~~27~~.0402 27.0402 27.0402
27.7824 27.7823 27.7752
+30.5408 (7.118) 30.7790 (.46) 30.9680
2000 / 6000 / 2000
1508/ml x3 / 450x/ml
2.7584 2.9967 3.1928
+.3 5.95 .84 +.3 7.12 6.40 .72 +.3
Sol m 3.0584 6.80 3.2967 3.4928
Coll 2.89 3.11 3.30

7.12 6.79 .33 .21

C-190 12/21

SUBJECT

SHEET NO.

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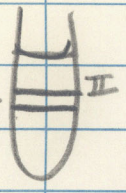
Cs Cl run – each tube had 2 red bands each fluorescent in UV light



I & II collected by drops (A) or by past pipette (B).

Eth. Br. extr. ~ isopropanol ~ equal vol X 3. + DNA sol'n dial ag 2 L 1/10 SSC 10^{-4} M EDTA X 2. in cold.

Count. 025 ml ^{14}C DNP fr.

		Total vol	Total cts	
I	416 cpm	~.7 ml	~2800	)
II	625 "	~1 ml		)
bet I & II	33 "			

C-190 12/20 Cleavage of SV40 DNA by cell extracts SHEET NO.

SUBJECT Prep. of extracts (Sambrook + Thatcher J Virol 1969) BY DATE

Cells: 1. CV-1 MEM 10% FBS to confluence. 12/20
Fed 24 hrs ā washing + freezing
✓ 2. SV 3T3 as above confl.
3. 3T3
✓ 4. ~~MA~~ 196 - Human skin diploid fr. W. Carter - ~2/3 confluent
2/5/70 ✓ 5. Lcells - (fr. W. Carter) confl (2/3/70) - 5 dishes (100 mm)

Extract Wash cells on disk ē ice cold PBS x2, then TED (.02M Tris Cl pH 8, 0.001 EDTA, 0.5 mM DTT) x1. Collect ē rubber policeman + store at -70° in 0.2 ml batches. Before assay thaw + cent at 7000 g for 10' at 0°. Use supe

Lysate assay conditions:
0.1M KCl, 0.04M Tris 7.7, 0.01M $MgCl_2$
0.01M ME 10^{-4}M ATP Vol = 0.12 ml
(~10% prot.) Stopped by addn EDTA.

.5 of .2
.1/.2

C-190 12/29/69 Hemophilus R enz. on SV40 ^{14}C-DNA for electrophoresis

SUBJECT

SHEET NO.

BY

DATE

Buffer mix	10X			
			10 ml	
	Tris HCl 7.4	0.10 M	1 ml 1M	
	$MgCl_2$	0.09 M	.9 ml 1M	
	ME	0.07 M	.05 ml 14M	
	NaCl	0.4 M	.8 ml 5M	
			7.25 water	
	1	2		
Buffer mix	.005	.005		
Water → .05	.005	.003		
DNA I ^{14}C prep B 12/69	.04	.04	(~ 8100 cpm)	
Enzyme	.0	.002		

f. AS 12/22/69 10+12

35° x 30' Then add 20 l 0.2M EDTA pH 7.4 + .01% sucr.

Electrophorese entire amt. + add in 5 l 2.5M sucr = BPB

(Note: BPB blue in tube 1, yellow in tube 2!)

Buffer — Tris - NaAc - EDTA pH 7.8 + 0.2% SDS

Tube 1 — gel 2

Tube 2 — gel 5

4 ma amp/tube 6:15 PM to 7:45 AM 13½ hrs

5% gels — 10 cm length

~~Results~~ Gels frozen at −20° + sliced in 3 segments ea a 4 cm egg slicer (→ 32 slices)

Dips in 0.2 ml H_2O_2 at 70° overnight + usual Triton-

Results over scint fl added

Results

#2-1	320 cpm	#5-1	184 cpm
2	46	2	0
3	9	3	0
4	2	↓	↓
↓	0	↓	↓
79	↓	80	0
	0		

i.e only cts at origin in each case